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Glutamate Transporters in Neurologic Disease

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Glutamate is the primary excitatory amino acid neurotransmitter in the human brain. It is important in synaptic plasticity, learning, and development. Its activity at the synaptic cleft is carefully balanced by receptor inactivation and glutamate reuptake. When this balance is upset, excess glutamate can itself become neurotoxic.

The neurotoxic properties of glutamate were first demonstrated in 1957 by Lucas and Newhouse,[1] who showed that systemic administration of glutamate to infant mice caused retinal degeneration. Over the last 4 decades, a direct correlation between the neuroexcitatory and neurotoxic properties of glutamate has been linked to activation of excitatory amino acid receptors.[2-5] This overactivation leads to an enzymatic cascade of events ultimately resulting in cell death.

Regulation of synaptic transmission and glutamate levels in the synaptic cleft is performed by glutamate transporters. Glutamate transport is a sodium- and potassium-coupled process that is capable of concentrating intracellular glutamate up to 10000-fold compared with the extracellular space.[6,7] These transporters are located throughout the human central nervous system as well as other tissues. Recent physiologic studies provide evidence that glutamate transporters keep synaptic concentrations of glutamate low enough to prevent receptor desensitization and/or excitotoxicity. New insights into the biology of these transporters suggest that their dysfunction may contribute to neurologic disease.

HUMAN GLUTAMATE TRANSPORTERS

Both neurons and astroglia are capable of high-affinity, sodium-dependent glutamate transport.[8] To date, 5 high-affinity, sodium-dependent glutamate transporters have been cloned from mammalian and human tissue: astrocyte-specific glutamate transporter (GLAST [excitatory amino acid transporter 1 (EAAT1)]), glutamate transporter 1 (GLT-1 [excitatory amino acid transporter 2 (EAAT2)]), excitatory amino acid carrier 1 (EAAC1 [excitatory amino acid transporter 3 (EAAT3)]), excitatory amino acid transporter 4 (EAAT4), and excitatory amino acid transporter 5 (EAAT5) (**Table**).[9-14]

Immunohistochemical studies have revealed that EAAT1 and EAAT2 are localized primarily in astrocytes, while EAAT3 and EAAT4 are distributed in neuronal membranes. Detailed immunogold studies have further delineated the localization of glutamate transporters to certain subcellular compartments. The neuronal transporters EAAT3 and EAAT4 appear to be localized to plasma membranes in a perisynaptic distribution. The greatest density of these transporter proteins appears to be at the edge of postsynaptic densities, rather than within the synaptic cleft. To date, most immunolocalization studies have further indicated that the neuronal transporters are localized in a somatodendritic fashion on postsynaptic spines and somas. They are rarely found presynaptically. In fact, to date, the only localization of glutamate transporters presynaptically has been on presynaptic inhibitory γ-aminobutyric acid (GABA) terminals.[15]

In a similar fashion, the astroglial glutamate transporters also have a polarized distribution. Both EAAT1 and EAAT2 are

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Distribution of Mammalian Glutamate Transporters and Their Human Homologues*

Glutamate Transporter	Human Homologue	Cell Type	Distribution
GLAST	EAAT1	Astrocytes	High expression in cerebellum; less in brain and spinal cord
GLT1	EAAT2	Astrocytes	Widespread throughout brain and spinal cord
EAAC1	EAAT3	Neurons	Hippocampus, cerebellum, basal ganglia
EAAT4	EAAT4	Neurons	Purkinje cells of cerebellum
EAAT5	EAAT5	Neurons	Retina

**GLAST indicates astrocyte-specific glutamate transporter; EAAT1, excitatory amino acid transporter 1; GLT-1, glutamate transporter 1; EAAT2, excitatory amino acid transporter 2; EAAC1, excitatory amino acid carrier 1; EAAT3, excitatory amino acid transporter 3; EAAT4, excitatory amino acid transporter 4; and EAAT5, excitatory amino acid transporter 5.*

localized to astroglial membranes that immediately oppose synaptic cleft regions of the neuropil (**Figure 1**).[16] In mammalian studies, it has been demonstrated that EAAT1 is highly expressed in the molecular layer of the cerebellum and moderates activity in the hippocampus, superior colliculus, and substantia gelatinosa of the spinal cord. In contrast, EAAT2 expression is generally high throughout all brain regions and the spinal cord but is largely absent from white matter tracts; EAAT3 is selectively enriched in neurons of the hippocampus, cerebellum, and basal ganglia; EAAT4 is largely confined to the soma and dendrites of the Purkinje cells of the cerebellum; EAAT5 is located in retinal ganglion cells (Table).

Thus, the anatomic analysis of the molecular subtypes of glutamate transporters suggests that glutamate inactivation may be either postsynaptic or on astroglial membranes. In fact, in the hippocampus, a region of intense glutamatergic innervation, there is little evidence for presynaptic or postsynaptic inactivation by neuronal transporters. Rather, all available data suggest that astroglial transporters are the predominant physiologic pathway for synaptic inactivation of glutamate in the forebrain.

NEUROSCIENTIFIC STUDY OF GLUTAMATE TRANSPORTER DYSFUNCTION

How does glutamate transporter dysfunction lead to neurotoxic effects and subsequent neurologic sequelae? The relationship between loss of glutamate transporters and enhancement of extracellular glutamate levels with subsequent neurotoxic effects has been well established.

Knockout mice deficient in the glutamate transporter subtypes have been developed. They yield a variety of phenotypes, including seizures, loss of motor coordination, and disturbances in amino acid metabolism.[17,18] The knockout-mouse model allows for the study of glutamate and its transporters throughout the development of the mammalian brain.

A second method in examining the effects of loss of glutamate transport is the use of antisense oligonucleotides to reduce the number of glutamate transporters in adult animals. Antisense oligonucleotides are believed to exhibit their effect by binding to the target messenger RNA (mRNA) and preventing its translation into the target protein. The infusion of these molecules over days to weeks simulates the chronic loss of transporters that may occur in neurodegenerative disorders. Reduction in various subtypes of glutamate transporters has led to models of amyotrophic lateral sclerosis (ALS) and epilepsy by increasing glutamate in the synaptic cleft and producing subsequent neurotoxic effects.

Cell culture systems have provided new evidence that supports the participation of reactive oxygen species (peroxynitrite, among others) in inhibiting glutamate transporter activity.[19] This inhibition leads to increased extracellular glutamate, which, through the activation of glutamate receptors, generates a cascade of enzymatic steps that further enhance the formation of reactive oxygen species.

Finally, models of hypoxia have been generated that show that depletion of adenosine triphosphate levels leads to the rundown of glutamate transport and actually leads to reversed uptake and the extrusion of glutamate into the synaptic cleft. This process further induces glutamate neurotoxic effects and may play a role in enhancing cell death (**Figure 2**).

Whether loss of glutamate transporter function is the primary insult or part of a cascade leading to neuronal death, it is becoming increasingly clear that glutamate transporters play a role in neurologic disease.

GLUTAMATE TRANSPORT AND HUMAN DISEASE

Amyotrophic Lateral Sclerosis

Multiple mechanisms have been postulated to cause motor neuron degeneration in sporadic and familial forms of ALS, including excitotoxic effects, oxidative injury, cytoskeletal abnormalities, and autoimmunity. It is likely that multiple primary insults result in the common phenotype of ALS. Evidence for glutamate contributing to motor neuron degeneration in ALS initially came from several studies that suggested that cerebrospinal fluid glutamate levels may be elevated in patients with sporadic ALS.[20,21] These earlier studies reported that motor cortex and spinal cord tissue glutamate levels were decreased 30% to 45% in patients with ALS. These alterations in extracellular and tissue glutamate may in fact reflect alterations in glutamate transport. This hypothesis was subsequently evaluated and confirmed through the use of membrane preparations of postmortem tissue from ALS patients and controls. In those studies, a significant loss of high-affinity, sodium-dependent glutamate transport was found in ALS.[22] Detailed studies were performed to examine molecular subtypes of glutamate transport in ALS. These revealed that up to 60% to 70% of patients with sporadic ALS have a 30% to 90% loss of the EAAT2 protein, in both motor cortex and spinal cord.[23] The loss of EAAT2 appears to be specific to these regions

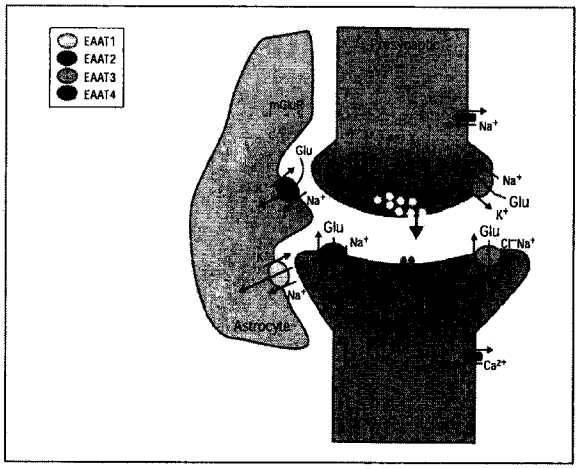

Figure 1. *Cellular localization of glutamate (Glu) transporter subtypes: EAAT1 and EAAT2 are found in the perisynaptic region of astroglial membranes; EAAT3 and EAAT4 are localized to neuronal membranes. mGluR indicates metabotropic glutamate receptor; K, potassium; Na, sodium; Cl, chlorine; NMDA, N-methyl-D-aspartate; and AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid. See the Table footnotes for an explanation of EAAT1 through EAAT4.*

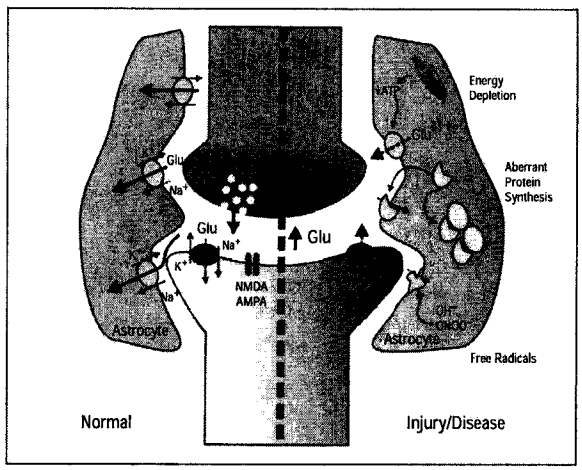

Figure 2. *Under normal conditions, glutamate (Glu) released into the synaptic cleft is removed (thick solid arrows at left) by sodium (Na)-dependent neuronal and astroglial Glu transporters (red and yellow ovals). Increased Glu at the synapse can result from the reversal of Glu transport (dashed arrows) under conditions of adenosine triphosphate depletion (ischemia). Truncated Glu transporters (incomplete ovals) may interact with full-length transporters to be sequestered within the cell or trafficked to the membrane, where they function ineffectively. Reactive oxygen species ($ONOO^-$, OH^-) generated by a variety of conditions may damage transporters (withered oval), with a resultant reduction in Glu transport. K indicates potassium; Na, sodium; Cl, chlorine; NMDA, N-methyl-D-aspartate; ATP, adenosine triphosphatase; and AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.*

in most but not all patients. This loss of EAAT2 protein cannot be attributed to cell death since there is no significant astroglial loss in ALS.

Parallel with human studies, a number of laboratories have been investigating the biology of the EAAT2 protein. Functional studies have determined that the EAAT2 transporter is the most abundant glutamate transporter in the brain, both at the protein level and functionally. Up to 95% of all tissue glutamate transport appears to be through the EAAT2 glutamate transporter.[17]

What is the relevance of a loss of EAAT2? Both in vitro and in vivo studies have documented that antisense knockdown or pharmacologic inhibition of a glutamate transporter leads to neuronal degeneration, especially of the motor neurons. In adult animals, antisense knockdown of EAAT2, analogous to an adult-onset loss of EAAT2 in ALS, leads to progressive paralysis in motor neuron degeneration.[24] Thus, the loss of EAAT2 protein is sufficient to induce a phenotype of motor neuron degeneration.

What could cause the loss of an astroglial glutamate transporter in a regional manner in sporadic ALS? Two possible mechanisms for loss of glutamate transporter proteins in ALS have been suggested. First, studies in ALS have revealed the presence of truncated RNA species in patients with sporadic ALS. Detailed analyses have revealed that ALS is associated with a large increase in multiple aberrant RNA species that code for truncated versions of the EAAT2 protein. Although 1 or 2 of these species can occasionally be seen in control specimens, ALS is unique in both the abundance, using vigorous quantitative methods to assess these truncated RNA species, and the large number of different truncated RNA species in individual patients.[25] Studies of some of these truncated species indicate that they have a dominant-negative effect on the EAAT2 protein and provide a mechanism for explaining a loss of EAAT2 protein in patients.

Second, evidence suggesting a link between free radical formation and glutamate transporter dysfunction comes from a mouse model of ALS. Mutations of superoxide dismutase (*SOD1*) have been found in approximately 10% of patients with familial ALS.[26] Transgenic mice overexpressing mutant *SOD1* genes display a slowly progressive motor neuron disease resembling ALS.[27] The mechanism for the neurotoxic effects associated with mutant *SOD1* is not yet known, but evidence supports the gain of a toxic property.[28-30] In addition, recent studies have documented that mutant *SOD1* by itself can induce oxidative damage to the EAAT2 protein that could also provide an alternate means for loss of glutamate transport in ALS patients.[31] Regardless of the mechanism, the loss of EAAT2 glutamate transporter may contribute to a reduction in glutamate uptake with subsequent overstimulation of glutamate receptors, resulting in neurotoxic effects.

As described above, glutamate transporters may be a target for these toxic effects. In fact, recent studies of *SOD1* transgenic mice show a marked loss of GLT1 (EAAT2) in the spinal cord as well as a loss of functional glutamate transport.[32] Thus, the loss of glutamate transport is seen both in familial models of ALS and in sporadic disease.

Alzheimer Disease

The neurodegeneration in AD is characterized by synaptic and neuronal loss with plaque and tangle formation. Abnormal expression or pro-

cessing of growth-associated proteins in the central nervous system may play a role in the process, leading to damage and neurodegeneration. Amyloid precursor protein has been implicated as being important in the pathogenesis of AD. Recently, it has been demonstrated that abnormal processing of amyloid precursor protein may be associated with the deficient functioning of the glutamate transporter system. In fact, a fragment of β-amyloid (Aβ), the central constituent of neuritic plaques in AD, inhibited tritium-labeled glutamate uptake in cultured astrocytes. Since reactive oxygen species are mediators of Aβ toxic effects and uptake inhibition by Aβ was prevented by antioxidants, it is conceivable that, among other effects, Aβ produces glutamate transporter oxidation and dysfunction.[33]

Stroke/Ischemia

Aberrant function of glutamate transport plays an essential role in the excitotoxic neurodegeneration that occurs in models of cerebral ischemia. As mentioned in the introduction, there is a tenfold higher concentration of glutamate within cells compared with the outside environment. The energy and ion gradient necessary to maintain this state fail under ischemic conditions. In fact, numerous in vitro studies have documented the actual reversal of glutamate transporter: glutamate that runs down its gradient from within cells to swamp the extracellular environment with large amounts of intracellular glutamate.[34-36]

Changes in glutamate transporter expression are seen with cerebral ischemia in animal models and human tissue. Astrocyte-specific glutamate transporter expression was increased in the penumbra 72 hours following ischemia in an animal model. This suggests that a compensatory increase in the activity of glutamate transporters may accompany pathological changes after ischemic injury.[37] The paucity of GLAST and GLT1 in specific regions of the hippocampus may account for the vulnerability of these neurons to an ischemic insult.[38]

Transient hypoxic-ischemic injury in a neonatal pig model demonstrates reduced levels of GLT1 and EAAC1 at 24 hours of recovery. Thus, astroglial and neuronal injury were found to occur rapidly in the newborn striatum, with early gliodegeneration and glutamate transporter abnormalities contributing to neurodegeneration.[39]

Selective cell vulnerability to neonatal hypoxia-ischemia may be attributed to loss of glutamate transporter subtypes. Changes in GLAST and EAAT4 (a Purkinje cell–specific transporter) in the cerebellum of hypoxic human neonates, examined postmortem, may account for the well-described vulnerability of Purkinje cells to hypoxic injury.[40]

While the regulation of the different transporter subtypes in varying anatomic regions and ischemic zones is still being studied, these changes are in response to and a result of neurotoxic effects.

Epilepsy

The family of glutamate transporter proteins may also be participants in certain models of epilepsy, although their role may be dependent more on their participation in the central nervous system metabolism than on their role as regulators of external glutamate concentrations. In knockout mice, a reduction in the glutamate transporter GLT1 results in lethal spontaneous seizures. By 6 weeks of age, 50% of animals die. Pathologically, some of the mice that lack the GLT1 transporter show destruction of neurons in the hippocampus, a region found to be important in the generation of seizure disorders.[17] Interestingly, developmental studies indicate that this time point is critical for the development of excitatory synapses. The loss of a predominant glutamate transporter in the neonatal brain, GLT1, therefore may be critical for normal synaptogenesis and prevention of seizures. In that regard, it is interesting that in adult animals, the loss of GLT1 leads not to seizures but, as described above, motor neuron degeneration. Thus, alterations in transporter expression may have pathophysiologic consequences for the cell types in which they are expressed, their ultrastructural localization, and the developmental timing at which insults occur. Interestingly, GLAST and EAAC1 knockout mice, while not normal, do not develop seizures.

In acquired models of epilepsy in which seizures are induced using a variety of pharmacological models, the data are somewhat conflicting. In a study of mRNA and protein expression using fully kindled rats, few changes in GLT or GLAST were found in the hippocampus.[41] Conversely, when the glutamate receptor agonist kainate was used to induce seizures, EAAC1 mRNA and protein levels were decreased in the rat hippocampus, GLT1 mRNA and protein levels were increased, and GLAST mRNA levels were increased.[42,43]

Recent experimental studies have provided a new means by which glutamate transporters may contribute to epilepsy. Infusing antisense oligonucleotides into the ventricles of adult rats with the molecular knockdown of EAAC1, a highly expressed hippocampal transporter, can produce episodic seizures in these animals.[24] Initial studies suggest that this effect occurs not through alterations of an extracellular glutamate, but rather through perturbations of the neurotransmitter GABA. The EAAC1 transporter is highly localized to GABA presynaptic terminals, and preliminary studies suggest that its dysfunction can alter neurotransmitter GABA metabolism (unpublished results from our laboratory). This alteration results in a loss of presynaptic release of GABA, diminishing inhibition. A disturbance of this metabolic function of glutamate transporters could underlie some pathophysiologic pathways of epilepsy.

In patients undergoing anterior temporal lobectomy for refractory seizures, brain tissue from the anterior temporal lobe did not reveal changes in the level of expression of the glutamate transporters EAAT1 and EAAT2.[44] In human studies of hippocampal sclerosis, however, EAAT2 and EAAT3 levels are increased in areas where neurons are spared and reduced in regions of neuronal cell loss.[45]

Taken together, these data suggest that alterations in glutamate transporters in both human tissue and animal models may play a role

in the generation and propagation of ictal activity. Determining whether these changes are the primary cause of induction of seizures or a compensatory response to neuronal injury requires further study.

APPLICATIONS FOR DIAGNOSIS

Currently, the World Federation of Neurology criteria are used to establish a diagnosis of ALS.[46] These criteria are based upon history and physical findings suggesting loss of upper and lower motor neurons and electrophysiologic evidence of denervation. Unfortunately, the diagnosis is often not established until late in the disease. New approaches to support the diagnosis are therefore welcome.

Lin et al[25] detected EAAT2 mRNA splice mutants in the cerebrospinal fluid of 66% of patients with sporadic ALS, but none in patients with nonneurologic disease or in controls with other diseases. Importantly, these splice mutants were also detectable early in the course of the disease. Although currently reliable qualitative and quantitative polymerase chain reaction methods might be difficult to perform in clinical laboratories, the collection of cerebrospinal fluid could be an adjunct to the current methods of diagnosis in the future. The identification of markers contributing to disease activity by conventional lumbar puncture may eventually lead to earlier diagnosis and institution of treatment for this devastating disease.

COMMENT

Glutamate neurotoxicity has long been known to contribute to the pathogenesis of neurologic disorders such as stroke, epilepsy, and ALS. The finding that glutamate transporter dysfunction plays a role in these disorders is a more recent discovery. Given that glutamate is ubiquitous in the central nervous system, glutamate transporter dysfunction may play a role in other neurologic disorders as well.

At the present time, several drugs used to treat neurologic disorders have activity at the glutamatergic synapse. Glutamate receptor antagonists have been tried in stroke in an attempt to limit the size and severity of ischemic insults. Riluzole is currently approved for use in the treatment of ALS and is believed to act by preventing the release of glutamate.[47] The antiepileptic drug topiramate acts as an antagonist of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)/kainate subtype of the glutamate receptor.[48]

Recently, a number of proteins have been identified that can modulate glutamate transporters.[49,50] These proteins appear either to potently stimulate or to inhibit glutamate transporter subtypes. Future manipulation of these proteins may also provide novel therapeutic means to regulate glutamate transport and afford therapeutic benefit.

Given what we have learned from the therapeutic applications of compounds active at glutamatergic synapses, manipulation of glutamate transporters may also prove promising. Future directions could include the development of glutamate transporter agonists to increase glutamate uptake from the synaptic cleft.

The use of gene therapy to deliver genes of interest to particular cell types is a rapidly expanding field. Gene therapy may be implemented to overexpress glutamate transporters in target cells. Glutamate transport from the extracellular space could be facilitated by increasing the number of glutamate transporters in neurons and glia.

The biology of free radical formation and its relationship to disease has garnered a great deal of attention recently. This has led to the pharmaceutical use of antioxidants to treat a host of different disorders. Antioxidants may be of use in preventing damage to glutamate transporters, offering an exciting approach to preventing glutamate accumulation in the synapse.

The study of these transporters as they relate to neurologic disease in humans is in its infancy. Understanding their biology will be critical in developing strategies for manipulating them in the future.

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REFERENCES

1. Lucas DR, Newhouse JP. The toxic effect of sodium L-glutamate on the inner layers of the retina. *Arch Ophthalmol.* 1957;58:193-201.
2. Olney JW, Ho OL. Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine. *Nature.* 1970;227:609-611.
3. Olney JW. Glutamate-induced neuronal necrosis in the infant mouse hypothalamus: an electron microscopic study. *J Neuropathol Exp Neurol.* 1971; 30:75-90.
4. Olney JW. The toxic effects of glutamate and related compounds in the retina and the brain. *Retina.* 1982;2:341-359.
5. Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science.* 1969;164:719-721.
6. Kanner BI, Schuldiner S. Mechanism of transport and storage of neurotransmitters [review]. *CRC Crit Rev Biochem.* 1987;22:1-38.
7. Nicholls D, Attwell D. The release and uptake of excitatory amino acids [review]. *Trends Pharmacol Sci.* 1990;11:462-468.
8. Hertz L. Functional interactions between neurons and astrocytes, I: turnover and metabolism of putative amino acid transmitters [review]. *Prog Neurobiol.* 1979;13:277-323.
9. Pines G, Danbolt NC, Bjoras M, et al. Cloning and expression of a rat brain L-glutamate transporter [published erratum appears in *Nature.* 1992;360: 768]. *Nature.* 1992;360:464-467.
10. Kanai Y, Hediger MA. Primary structure and functional characterization of a high-affinity glutamate transporter. *Nature.* 1992;360:467-471.
11. Storck T, Schulte S, Hofmann K, Stoffel W. Structure, expression, and functional analysis of a Na (+)-dependent glutamate/aspartate transporter from rat brain. *Proc Natl Acad Sci U S A.* 1992; 89:10955-10959.
12. Arriza JL, Fairman WA, Wadiche JI, Murdoch GH, Kavanaugh MP, Amara SG. Functional comparisons of three glutamate transporter subtypes cloned from human motor cortex. *J Neurosci.* 1994;14:5559-5569.
13. Fairman WA, Vandenberg RJ, Arriza JL, Kavanaugh MP, Amara SG. An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. *Nature.* 1995;375:599-603.
14. Arriza JL, Eliasof S, Kavanaugh MP, Amara SG. Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. *Proc Natl Acad Sci U S A.* 1997;94:4155-4160.
15. Furuta A, Martin LJ, Lin CLG, Dykes-Hoberg M, Rothstein JD. Cellular and synaptic localization of the neuronal glutamate transporters excitatory amino acid transporter 3 and 4. *Neuroscience.* 1997;81:1031-1042.
16. McDermott RH, Butler M. Uptake of glutamate, not glutamine synthetase, regulates adaptation of mammalian cells to glutamine-free medium. *J Cell Sci.* 1993;104:51-58.
17. Tanaka K, Watase K, Mabe T, et al. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science.* 1997;276: 1699-1702.

18. Peghini P, Janzen J, Stoffel W. Glutamate transporter EAAC-1-deficient mice develop dicarboxylic aminoaciduria and behavioral abnormalities but no neurodegeneration. *EMBO J.* 1997;16:3822-3832.
19. Trotti D, Rossi D, Gjesdal O, et al. Peroxynitrite inhibits glutamate transporter subtypes. *J Biol Chem.* 1996;271:5976-5979.
20. Rothstein JD, Tsai G, Kuncl RW, et al. Abnormal excitatory amino acid metabolism in amyotrophic lateral sclerosis. *Ann Neurol.* 1990;28:18-25.
21. Rothstein JD, Kuncl R, Chaudry V, et al. Excitatory amino acids in amyotrophic lateral sclerosis: an update [letter]. *Ann Neurol.* 1991;30:224-225.
22. Rothstein JD, Martin LJ, Kuncl RW. Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. *N Engl J Med.* 1992;326:1464-1468.
23. Bristol LA, Rothstein JD. Glutamate transporter gene expression in amyotrophic lateral sclerosis motor cortex. *Ann Neurol.* 1996;39:676-679.
24. Rothstein JD, Dykes-Hoberg M, Pardo CA, et al. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron.* 1996;16:675-686.
25. Lin CL, Bristol LA, Jin L, et al. Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter, in amyotrophic lateral sclerosis. *Neuron.* 1998;20:589-602.
26. Rosen DR, Siddique T, Patterson D, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature.* 1993;362:59-62.
27. Gurney ME, Pu H, Chiu AY, et al. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science.* 1994;264:1772-1775.
28. Ripps ME, Huntley GW, Hof PR, Morrison JH, Gordon JW. Transgenic mice expressing an altered murine superoxide dismutase gene provide an animal model of amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A.* 1995;92:689-693.
29. Zhang Y, Pines G, Kanner BI. Histidine 326 is critical for the function of GLT-1, a (Na+ + K+)-coupled glutamate transporter from rat brain. *J Biol Chem.* 1994;269:19573-19577.
30. Reaume AG, Elliott JL, Hoffman EK, et al. Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. *Nat Genet.* 1996;13:43-47.
31. Trotti D, Rolfs A, Danbolt NC, Brown RH Jr, Hediger MA. *SOD1* mutants linked to amyotrophic lateral sclerosis selectively inactivate a glial glutamate transporter. *Nat Neurosci.* 1999;2:427-433.
32. Bruijn LI, Becher MW, Lee MK, et al. ALS-linked *SOD1* mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with *SOD1*-containing inclusions. *Neuron.* 1997;18:327-338.
33. Masliah E, Alford M, DeTeresa R, Mallory M, Hansen L. Deficient glutamate transport is associated with neurodegeneration in Alzheimer's disease. *Ann Neurol.* 1996;40:759-766.
34. Billups B, Attwell D. Modulation of non-vesicular glutamate release by pH. *Nature.* 1996;379:171-174.
35. Szatkowski M, Barbour B, Attwell D. Non-vesicular release of glutamate from glial cells by reversed electrogenic glutamate uptake. *Nature.* 1990;348:443-446.
36. Storm-Mathisen J, Danbolt NC, Rothe F, et al. Ultrastructural immunocytochemical observations on the localization, metabolism and transport of glutamate in normal and ischemic brain tissue [review]. *Prog Brain Res.* 1992;94:225-241.
37. Kalra S, Arnold DL, Cashman NR. Biological markers in the diagnosis and treatment of ALS [review]. *J Neurol Sci.* 1999;165(suppl 1):S27-S32.
38. Fujita H, Sato K, Wen TC, Peng Y, Sakanaka M. Differential expressions of glycine transporter 1 and three glutamate transporter mRNA in the hippocampus of gerbils with transient forebrain ischemia. *J Cereb Blood Flow Metab.* 1999;19:604-615.
39. Martin LJ, Brambrink AM, Lehmann C, et al. Hypoxia-ischemia causes abnormalities in glutamate transporters and death of astroglia and neurons in newborn striatum. *Ann Neurol.* 1997;42:335-348.
40. Inage YW, Itoh M, Wada K, Takashima S. Expression of two glutamate transporters, GLAST and EAAT4, in the human cerebellum: their correlation in development and neonatal hypoxic-ischemic damage. *J Neuropathol Exp Neurol.* 1998;57:554-562.
41. Akbar MT, Torp R, Danbolt NC, Levy LM, Meldrum BS, Ottersen OP. Expression of glial glutamate transporters GLT-1 and GLAST is unchanged in the hippocampus in fully kindled rats. *Neuroscience.* 1997;78:351-359.
42. Nonaka M, Kohmura E, Yamashita T, et al. Increased transcription of glutamate-aspartate transporter (GLAST/GluT-1) mRNA following kainic acid-induced limbic seizure. *Brain Res Mol Brain Res.* 1998;55:54-60.
43. Simantov R, Crispino M, Hoe W, et al. Changes in expression of neuronal and glial glutamate transporters in rat hippocampus following kainate-induced seizure activity. *Brain Res Mol Brain Res.* 1999;65:112-123.
44. Tessler S, Danbolt NC, Faull RLM, Storm-Mathisen J, Emson PC. Expression of the glutamate transporters in human temporal lobe epilepsy. *Neuroscience.* 1999;88:1083-1091.
45. Niebroj-Dobosz I, Janik P. Amino acids acting as transmitters in amyotrophic lateral sclerosis (ALS). *Acta Neurol Scand.* 1999;100:6-11.
46. Brooks BR, for the Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial "Clinical limits of amyotrophic lateral sclerosis" workshop contributors. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. *J Neurol Sci.* 1994;124(suppl):96-107.
47. Martin D, Thompson MA, Nadler JV. The neuroprotective agent riluzole inhibits release of glutamate and aspartate from slices of hippocampal area CA1. *Eur J Pharmacol.* 1993;250:473-476.
48. Shank RP, Gardocki JF, Vaught JL, et al. Topiramate: preclinical evaluation of structurally novel anticonvulsant. *Epilepsia.* 1994;35:450-460.
49. Jackson M, Jin L, Dykes-Hoberg M, et al. Activation of excitatory amino acid transporter 4 (EAAT4) by two novel interacting proteins. Abstract presented at: 29th Annual Meeting of the Society for Neuroscience; October 24, 1999; Miami, Fla. Abstract No. 170.3.
50. Lin CLG, Orlov I, Dykes-Hoberg M, Jin L, Rothstein J. Allosteric modulation of neuronal glutamate transporter EAAC1 by a novel associated protein GTRAP-18. Abstract presented at: 29th Annual Meeting of the Society for Neuroscience; October 24, 1999; Miami, Fla. Abstract No. 170.4.